Chain-Length Dependence of the Electrophoretic Mobility in Random Gels

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The electrophoretic mobility μ of a flexible N-segment probe polymer has been measured in cross-linked gels of concentration c. Assuming $\mu \sim N^{-\alpha} c^{-\gamma}$, α initially increases with N, reaches a maximum of ~ 2 in a crossover regime, and finally decreases to 1, the exponent of reptation. In accord with a semidilute-solution scaling argument, an analogous maximum in γ is observed. Behavior at intermediate N and c is consistent with "entropic-barrier"-mediated transport, while larger N or c leads to reptation.

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Although much effort has been directed toward a molecular description of gel electrophoresis, the underlying mechanisms remain poorly understood even for simple polymer solutes. The reptation model provides one exception, successfully accounting for the motion of large linear DNA molecules in agarose gels [1] and perhaps the migration of proteins during sodium-dodecylsulfate poly(acrylamide)-gel electrophoresis (SDS-PAGE) [2]. Under reptation at low applied fields, the probe chain "snakes" its way through a "tube" representation for the gel; a simple analysis of this motion finds that the electrophoretic mobility μ is inversely proportional to the degree of polymerization N. Inasmuch as DNA molecules and protein-SDS complexes can be regarded as flexible linear solutes of uniform charge density, the success of the reptation approach appears reasonable. Because of their flexibility, both solutes adopt random coil conformations in which the statistical coil size R is given $R \sim N^{\nu}$, where ν is an excluded volume exponent (typically 0.5–0.6); this coiled conformation persists at low applied fields, even within a gel, but becomes highly distorted when fields are larger. The strong N dependence noted in gels at low fields must arise solely from coil-gel interactions, as several investigators have observed that the free-solution mobility μ_0 of a charged macromolecule is independent of N [3,4].

Many gel electrophoresis studies, particularly at lower molecular weight and/or in highly porous gels, have found that μ is not inversely proportional to N. We have recently proposed a general depiction in which the ratio of R to ξ, the mesh spacing, determines the operative transport mechanism [5]. When R/ξ is small, the charged coil moves rather freely through the matrix, with only occasional collisions with fixed gel sites; μ is therefore determined by the mutual excluded volume of a single coil and a single gel site. Across this unentangled regime, μ is principally governed by R [6]. When R/ξ is very large, on the other hand, motion is by reptation and μ is controlled by N. Spanning a broad crossover regime, where $R/\xi \sim 1$, we argued for the existence of transport directed by "entropic barriers," with N again the controlling variable.

The entropic-barrier mechanism was first suggested by Baumgartner and Muthukumar [7] to explain computer simulations of chain diffusion in random media, and the concept was subsequently developed more fully by the same authors using scaling arguments [8]. The basic premise is that a gel is spatially inhomogeneous and that the confinement entropy of a probe chain must therefore depend strongly on position. This entropy is greatest in relatively open "cavities" and lowest in constrictive "bottlenecks." For each center-of-mass position the entropy is calculated by counting all accessible conformations of the molecule. Repeating the calculation throughout the gel domain, a three-dimensional entropy surface is generated on which the center of mass moves under the combined influence of Brownian motion and the applied field. The chain's rate of movement is governed by the height, length, and frequency of constrictions imposed by the matrix. As R approaches ξ the confinement entropy becomes an extensive property, i.e., one that depends only on N and not on chain architecture. Therefore, μ no longer depends on molecular topology, a trend verified by experiment [5]. When the reptation regime is reached at still greater levels of confinement, chain topology strongly affects μ, and different topologies are readily separated. The transition to reptation or other alternate transport mechanisms that might operate in strongly confining gels is not implicitly accounted for in the entropic-barrier model.

In their Monte Carlo calculations Baumgartner and Muthukumar [7] observed that the tracer diffusion coefficient D scaled with N such that the exponent β, of the relationship $D \sim N^{-\beta}$, approached 3 at the largest N values simulated; this exponent significantly exceeds the one predicted by reptation ($\beta = 2$). Using scaling arguments appropriate to the entropic-barrier regime, it was later suggested by the same authors that β could increase indefinitely with N and that a power-law representation for $D(N)$ is inappropriate [8]. In recent experiments several investigators have observed $\beta > 2$ for self-diffusion in gels and concentrated polymer solutions [9]. With polymers of uniform charge density, μ can be related to D by the simple Einstein relationship $\mu \sim DQ \sim DN \sim N^{-\alpha}$, where Q is the total charge on the chain and $\alpha = \beta - 1$. One therefore expects that μ in the entropic-barrier re-

gime could exhibit a greater N exponent than in the terminal, highly confined regime ($\alpha=1$); on a log-log plot, the $\mu(N)$ relationship would then appear sigmoidal. Surprisingly, such behavior has not been reported, and in fact, is not observed with either poly(styrene sulfonate) (PSS) or DNA in agarose gels [1,10]. Agarose gels are poorly characterized as to their microscopic structure, so in the present contribution we describe efforts to measure $\mu(N)$ in a better defined gel system comprised of a chemically cross-linked network.

PSS was selected as the test polymer because of its high inherent flexibility and our ready access to a wide range of narrow and well-defined molecular-weight samples ($38<N<9800$); chain stiffness is controlled, to some degree, by the ionic strength of the solvent. We have previously described an extensive array of PSS electrophoresis experiments conducted with agarose gels; in general, PSS behaviors were found similar to those of high-molecular-weight DNA [10]. In the present investigation the medium is cross-linked poly(acrylamide), a material conventionally employed to fractionate proteins but applied in one previous study to fractionate PSS [11]. A poly(acrylamide) gel is prepared by adding an appropriate amount of a 29:1 acrylamide–bis-acrylamide mixture to an aqueous solution containing the initiator (ammonium persulfate and tetramethylethylenediamine) and sufficient Na_2HPO_4 to adjust the final ionic strength to $0.03M$. The gel is cast as a slab between two glass plates, and electrophoresis is conducted in the vertical mode. Visualization of PSS bands is achieved at the end of a run by staining the gel with an aqueous solution of methylene blue (0.01% by weight), a cationic dye. The gel weight fraction c varies between 0.03 and 0.07, a range bounded on the low end by the mechanical weakness of a dilute gel and on the high end by the manageable duration of an electrophoresis run (several days). The imposed field strength, 0.765 V/cm, is so low that μ is independent of field strength for all values of c and N examined; this independence has always been confirmed by duplicate measurements at lower field strengths.

The N dependence of μ is displayed in Fig. 1, with c as a parameter. The mobility clearly does not follow a power law in N for any extended range of N. At low N the mobility approaches its free-solution value as N is extrapolated toward 0. Fitting a slope to the steepest portion of each curve, α_{max} varies from ≈ 2.1 to ≈ 2.4. Although the data are limited, it appears that α approaches 1.0 in the asymptotic limit of large N. At $c=0.07$, in fact, a best fit to the five terminal N values provides $\alpha=1.02\pm0.04$; at this same gel concentration $\alpha_{max}\approx 2.4$, achieved at N just below the onset of the reptation power law. The sigmoidal curve shapes, with slopes exceeding -1 at intermediate N, are highly suggestive of entropic barriers as a crossover transport mechanism. The steepest region of each curve occurs at $R/\xi\approx 1$ [12,13], another feature supportive of the model. More rigorous comparisons to the theory are not possible, given

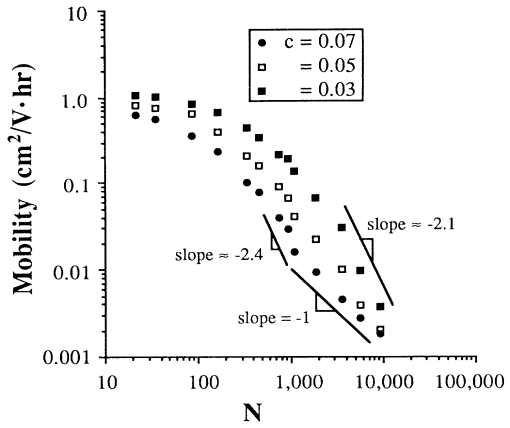

FIG. 1. Low-field mobility μ against chain length N for PSS in poly(acrylamide) gels. For each gel concentration c the slope exceeds -1 over an intermediate range of N; for the two higher concentrations the slopes go through a maximum before achieving large-N asymptotes close to the reptation value of -1. The lines drawn on the figure are for visual guidance and should not be regarded as fits to the data.

the qualitative nature of the current model [7,8] and our relative ignorance of the local matrix structure. The transition to reptation with increasing N is beyond the predictive capability of the entropic-barrier approach, which incorporates only the thermodynamics of confinement in the matrix; reptation is an independent form of chain transport not rooted in these thermodynamics.

Invoking the plausible assumption [14] that the poly(acrylamide) gel structure is similar to the solution structure of this polymer at equal concentration c, the mesh spacing will follow $\xi\sim c^{\nu'/(1-3\nu')}$, where ν' is the excluded volume exponent of poly(acrylamide) in water ($\nu'\approx\frac{3}{5}$ [15]); under the selected experimental conditions the excluded volume exponent ν of the mobile PSS probe chains is also about $\frac{3}{5}$ [16]. Combining this information and asserting [14] that the dimensionless mobility ratio μ/μ_0 depends only on R/ξ, the following relationships are developed: $\mu\sim N^{-\alpha}c^{\alpha\nu'/\nu(1-3\nu')}\sim N^{-\alpha}c^{-1.25\alpha}$. When $\alpha=1$, as for reptation, the predicted c exponent is -1.25. Figure 2 shows μ as a function of c for three selected values of N; at the largest N the -1.25 exponent is consistent with experimental data. At intermediate N the c dependence is greater, again as expected, although an insufficient amount of data prevents quantitative analysis. Finally, at the smallest N, the c dependence nearly disappears. Scatter in the data of Fig. 2 is unavoidable, as each value of c requires preparation of an entirely new gel, a process that is not perfectly reproducible. The experimental trends as c is varied are entirely consistent with semidilute-solution scaling arguments and the reported dependence of μ on N: In the entropic-barrier regime at intermediate N and c, the N and c dependences of μ are greatest. Molecular biologists frequently determine polymer size from the slope of semilogarithmic plots

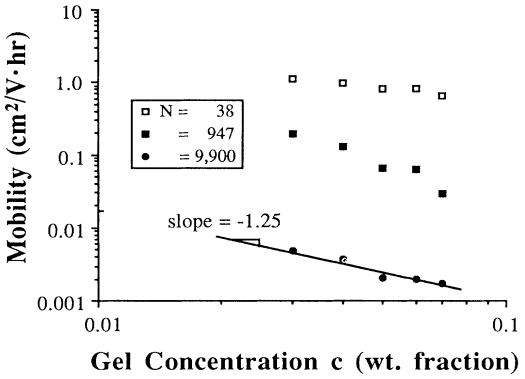

FIG. 2. PSS mobility against poly(acrylamide) gel concentration. The slope at intermediate N is the greatest, while the -1.25 slope at the larger N value follows from a combination of scaling arguments and reptation. Both trends are consistent with the N dependence of Fig. 1.

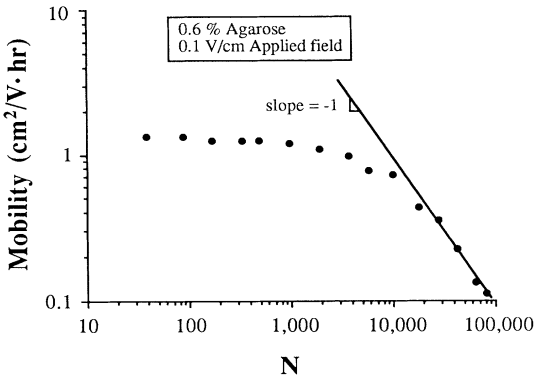

FIG. 3. The N dependence of μ for an intermediate-concentration agarose gel. The initial and terminal regions defined by Fig. 1 are clearly present, but the slope maximum at intermediate N is absent.

of μ vs c (known as Ferguson plots [17]); the present study clearly demonstrates the futility of this exercise when applied to flexible polymers.

Figure 3 shows the N dependence of μ in 0.6% agarose, a gel medium which competes with poly(acrylamide) in electrophoresis applications [10]. Although the matrix concentration is lower than in the gels previously discussed, the same transport regimes are traversed as N increases. In particular, the $R \ll \xi$ and $R \gg \xi$ behaviors are clearly reflected at small and large N. Surprisingly, at intermediate N there is no range over when the exponent α exceeds 1.0. Our previous study using chain topology as a variable, however, clearly demonstrated that over this N range a broad entropic-barrier regime exists in these gels [5]. The observation of $\alpha \leq 1$ for all N must therefore not be taken as conclusive evidence against the entropic-barrier model.

Entropic-barrier transport apparently plays a major role in the electrophoretic fractionation of flexible polymers in gel media, providing an effective mechanism for the fractionation of flexible chains by N at low applied fields. Large α exponents, reported here for the first time, cannot be explained to our knowledge by any other theory for gel electrophoresis. The unexpected difference between agarose and poly(acrylamide) gels suggests that an accurate structural characterization of the separation media will be necessary before an entropic-barrier theory can be applied quantitatively. The important structural difference between poly(acrylamide) and agarose gels can only be the subject of speculation, but one might expect that poly(acrylamide) gel matrices are more statistical due to their chemical synthesis.

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